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Tuberculosis outbreak in a grammar school, Serbia, 2016

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Abstract
Serbia has a low incidence of tuberculosis (TB), with a decreasing trend in the last decade. The purpose of this manuscript is to describe an outbreak of TB infection that occurred in 2016 among students of a grammar school in Novi Pazar. A 17-year-old girl, third-grade student of a grammar school (the index case), was diagnosed with smear-positive tuberculosis. Contact investigation was conducted, including chest X-ray examinations of over 1100 persons. After the index case was detected, a total of 16 (10 pulmonary and 6 extrapulmonary) tuberculosis patients were newly diagnosed during 2016. Among 11 culture positive cases, MIRU-VNTR method revealed that all *Mycobacterium tuberculosis* isolates were identical. Diagnostic delay contributed to the transmission of infection.

Key words
- tuberculosis
- outbreak
- grammar school

INTRODUCTION

Serbia has a low incidence of tuberculosis (TB); in 2016, incidence of notified cases was 11.8/100 000 population, with a decreasing trend in the last decade [1, 2]. However, higher rates were reported in some areas: the highest incidence of notified cases was reported in Raska District – 29.0/100 000 population in 2016, with an unfavorable trend in the last decade [2]. The BCG vaccine coverage in newborns was over 95%. Novi Pazar is the largest city located in the Raska District (the urban area has about 66 000 inhabitants, while the city administrative area has about 100 000 inhabitants). The grammar school of Novi Pazar has about 1200 students (in grades I-IV, with 40 classes) and around 100 employees. We described an outbreak of TB infection that occurred in 2016 among students of a grammar school in Novi Pazar.

METHOD AND MATERIAL

In this study, descriptive epidemiological method was used. This study was performed in Novi Pazar (a city in Serbia) during 2017. As a source of data in this paper, the material obtained during the investigation of the epidemic was used.

Case definition

A "TB case" was defined according to the diagnostic criteria proposed by the World Health Organization (WHO) [3], and implemented in Serbian guidelines for definition and classification of tuberculosis [4]. A case of TB denotes a patient in whom tuberculosis has been bacteriologically confirmed, or has been diagnosed by a specialist doctor. A definitive case of TB denotes a patient with positive culture for *Mycobacterium tuberculosis* (MTB) or a patient with two sputum samples positive for acid-fast bacilli. Cases were classified as pulmonary TB (the disease involves lung parenchyma and tracheobronchial tree) and extrapulmonary TB (the disease involves any organs other than the lung, including pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges etc., if lung parenchyma is not affected at the same time). A patient who at the same time has both pulmonary and extra-pulmonary TB was classified as a case of pulmonary TB. In absence of bacteriological confirmation of clinical specimens, TB diagnosed on the basis of clinical signs and symptoms alone, and by a radiologic, pathologic, or therapeutic response, and/or a positive tuberculin skin test that was consistent with active tuberculosis.

Case detection

Detection and identification of mycobacteria using classical, conventional methods is performed in accordance with national recommendations in referent laboratories [5]. All of the isolates were obtained from cultures of respiratory samples. Diagnosis was based on

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direct microscopic detection of acid-fast bacilli in sputum or other samples and isolation of MTB on appropriate medium, or on a pathohistological confirmation based on the finding of MTB in the histological sample and / or cultivation of the biological material obtained by a biopsy. Samples for direct microscopic examination were stained with carbol-fuchsin technique according to Ziehl-Neelsen. Processed samples were seeded on the Löwenstein-Jensen medium. Identification of MBT in samples was carried out using the GenoType® Mycobacterium MTBC (Hain Lifescience) assay. Conventional methods for testing the susceptibility of TB isolates were based on the detection of growth on solid substrates containing antituberculotics [4]. A proportion method, on modified Löwenstein-Jensen solid substrate, was used to examine the sensitivity of TB isolates to first-line anti-TB drugs. The susceptibility of TB isolates was examined for the following drugs: isoniazid, rifampicin, etambutol, streptomycin, pyrazinamide [5]. All TB isolates were susceptible to first line drugs. Response to first-line drug combination of anti-TB therapy was favorable and after some months *MTB* was not detectable on sputum microscopy.

Genomic investigation of Mycobacterium tuberculosis

To determine a detailed picture of tuberculosis epidemiology, all MTB isolates were analyzed by classical genotyping techniques, i.e. mycobacterial interspersed repetitive unit – variable number tandem repeat (MIRU-VNTR) genotyping on 24 loci [6]. MIRU-VNTR genotyping was performed in accordance with the protocol described by Supply *et al* [5]. The chromosomal DNA of the laboratory reference strain MTB H37Rv (ATCC 27294) was used as a control. The MIRU-VNTRplus database (https://www.miru-vntrplus.org) was used for identification by similarity search of the isolated strain [7]. Categorical distance was used as distance measure for strain comparison. The distance cutoff of 0.17 was used for identification.

Epidemiological investigation

Contact investigation was conducted in accordance with guidelines of the European consensus on the tuberculosis contact investigation in low prevalence countries [8]. From June 2016, as part of the investigation by local public health authorities for TB control, additional testing extended to all students who attended the same school during 2016, and to all school staff members. Testing had to be completed before the end of the school term. Contact investigation included chest X-ray examinations of over 1100 persons (students, employees of grammar school, as well as all family members of cases). Contact investigation revealed 10 new cases of pulmonary and 4 new cases of extrapulmonary tuberculosis in third-grade students. There were no tuberculosis cases among family members of sick students and among school staff. Additionally, contact research that included all staff of cafes and grocery shops near the grammar school, recorded absence of the disease among them.

RESULTS

In February 2016, TB pleuritis was identified in a 17-year-old girl who was a third year grammar school student. She experienced fatigue, hard breathing, dyspnea and weight loss. Patient was treated by a pediatrician, but, one month later, she was hospitalized because the severity of symptoms had increased, and MTB was isolated from a pleural fluid sample.

Between April and May of 2016, three students who had attended the same grammar school as the first case-patient, developed pulmonary tuberculosis. In these cases, tuberculosis was confirmed by isolation of MTB. By the end of December 2016, a total of 17 cases of TB were identified among students in the same school *(Table 1)*, including 10 pulmonary forms confirmed bacteriologically, 1 pulmonary form confirmed clinically without MTB isolation, 1 extrapulmonary form confirmed bacteriologically, 2 extrapulmonary forms confirmed pathohistologically and 3 extrapulmonary forms confirmed clinically. Except one case, all patients were students of the third grade of the grammar school. There were no tuberculosis cases among grammar school employees.

BCG scar was recorded in 13 cases. Only a few patients gave information that they smoke cigarettes, use nargile and consume alcohol. Positive family history for TB in the past was recorded in 4 students. Comorbid-

Table 1
General and clinical characteristics of the tuberculosis (TB) cases

Characteristics	Number (17)	%
Sex		
Male	7	41.2
Female	10	58.8
Grade		
III	16	94.1
II	1	5.9
Symptoms		
Cough	6	35.3
Fever	6	35.3
Fatigue	4	23.5
Night sweating	4	23.5
Breathing difficulty	3	17.6
Weight loss	6	35.3
Chest pain	3	17.6
Without symptoms	2	11.8
Comorbidity	0	0.0
Body Mass Index (<18.5 kg/m^2)	0	0.0
Habits		
Cigarettes smoking	1	5.9
Nargile use	2	11.8
Alcohol use	2	11.8
Positive family history for TB in past years	4	23.5
Contact with colleagues - cases with TB	17	100.0
Clinical findings		
Pulmonary TB	11	64.7
Extrapulmonary TB	6	35.3
BCG scar	13	76.5
MIRU-VNTR*		
Identical strains	11	100.0

MIRU-VNTR = mycobacterial interspersed repetitive unit - variable number tandem repeat; *Only for culture-confirmed TB.

Table 2
MIRU-VNTR profiles of control strain *Mycobacterium tuberculosis* H37Rv, all strains from Novi Pazar and strain identified through the similarity search of the MIRU-VNTRplus database

	MIRU loci																						
Strain	154 MIRU02	424 Mtub04	577 ETRC	580 MIRU04	802 MIRU40	960 MIRU10	1644 MIRU16	1955 Mtub21	2059 MIRU20	2163b OUB11b	2165 ETRA	2347 Mtub29	2401 Mtub30	2461 ETRB	2531 MIRU23	2687 MIRU24	2996 MIRU26	3007 MIRU27	3171 Mtub34	3192 MIRU31	3690 Mtub39	4052 OUB26	4156 OUB4156	4348 MIRU39
H37Rv	2	2	**4**	**3s**	**1**	**3**	**2**	**2**	2	**5**	**3**	4	**2**	**3**	**6**	1	**3**	3	3	3	**5**	5	**2**	2
Novi Pazar	2	2	3	2	3	5	3	1	2	2	2	4	4	2	5	1	5	3	3	3	4	5	3	2
Haarlem, Germany*	2	2	3	2	3	5	3	**3**	2	**4**	**3**	4	4	2	5	1	5	3	3	3	**3**	5	3	2

Abbreviations: MIRU-VNTR = mycobacterial interspersed repetitive unit – variable number tandem repeat.
*Categorical distance < 0.17 (Locus differences compared to the isolated strain are bolded).

ity (immunosuppressive conditions, malignant diseases, silicosis, renal insufficiency, diabetes mellitus, malnutrition) was not observed in patients.

All *Mycobacterium tuberculosis* isolates from students with culture-confirmed TB, analyzed by MIRU-VNTR genotyping techniques, revealed that all 11 strains from Novi Pazar were identical *(Table 2)*. Search of the MIRU-VNTR plus database showed that the MIRU-VNTR type identified in all cases in this epidemic in Serbia matched in 20 loci with a strain which represents the Haarlem sublineage (distance 0.1667) and which was isolated in Germany.

DISCUSSION

We described a high school TB outbreak among students in Novi Pazar, Serbia. Genotyping of *Mycobacterium tuberculosis* isolates of all cases confirmed that all cases in this outbreak were infected with the same strain.

The majority of cases (94.1%) were in the same year at school, which is in concordance with previous findings from a retrospective study of a TB outbreak in one secondary school in the United Kingdom, where a significantly higher risk for being diagnosed with TB was noted in pupils in the same school year as the first case-patient [9]. Previous studies suggested that the risk to school employees teaching children with TB might be very small, similar to what our study showed – no cases were detected among employees in the grammar school [9, 10]. Testing an entire high school for TB is uncommon, although a similar event occurred at a Colorado school in 2011, where 1249 persons were screened [10], alike to the outbreak we reported here, where local public health authorities examined over 1100 persons including students, staff of the grammar school and family members of cases. Investigation of a TB outbreak in a boarding middle school in China found significant efficacy of the vaccine [11], while we recorded a BCG scar in 76.5% of cases in this outbreak, indicating that previous BCG vaccination did not reduce the risk of TB in those cases.

No cases with this MIRU-VNTR genotype had been detected in Serbia prior to this: this MIRU-VNTR genotype suggests the Haarlem sublineage which belongs to the Euro-American lineage which is widespread across the Americas, Europe and Africa [12]. Search of the MIRU-VNTRplus database, as well as a search of the available literature showed that the MIRU-VNTR type identified in all cases in this epidemic in Serbia has not been previously reported as responsible for other TB cases or outbreaks in Serbia or abroad, either in young or adult persons [8, 12]. Our findings could be due to information lacking in the databases or could reflect the unique nature of MTB isolates from Novi Pazar. Because this TB outbreak consisted of a significant number of cases in otherwise healthy young people, more research is needed to obtain a better insight in the TB situation in Novi Pazar.

Risk factors for outbreaks of TB in schools involve constant contact, inadequate ventilation and delay in diagnosis [13, 14]. In this study, the delay in making a diagnosis led to postponement in treatment, which likely was a factor contributing to the TB outbreak [15, 16].

Epidemiological TB contact investigation established direct contact between all cases. Although the source of this outbreak remained unknown, the spread of the disease in grammar school can be linked to high TB incidence in the community, as well as the students' contacts in school and surrounding cafes. Genotyping of MTB isolates of all culture-confirmed cases showed that all of the cases in this outbreak were infected with the same strain.

CONCLUSION

This outbreak indicates the need for timely identification of TB and adequate treatment. Our findings emphasize the need for increasing the TB knowledge and awareness among community members, especially adolescents, as well as health professionals.

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Author contributions

All authors equally contributed to this paper with conception and design of the study, data collection, data analysis, interpretation of the results, manuscript preparation, critical revision and editing. All authors read, reviewed and approved the final manuscript.

Ethical considerations

This study is a part of a larger research approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac (Ref. No.: 01-1176).

Conflict of interest statement

The authors declare that they have no competing interests.

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